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EFFECT OF PASTEURIZATION ON MOLD SPORES

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INTRODUCTION

Definite experiments to determine whether spores of the common saprophytic molds survive the temperatures used for the pasteurization of milk have not been reported. These spores are certainly present and are frequently abundant in ordinary market milk. Vague and general statements that such organisms do or do not survive are not uncommon, but are not supported by reference to actual work. To obtain such data studies were made with spores from pure cultures of a series of molds including several species of *Penicillium*, *Aspergillus*, and of the mucors, with, in some experiments, the addition of *Oidium (Oospora) lactis* and one strain of *Fusarium*. These sets of experiments were made to test, as carefully as laboratory conditions would permit, the temperatures used in pasteurization by the "holder" process, those used in the "flash" process, and the effects of dry heat.

EXPERIMENTS WITH THE HOLDER PROCESS OF PASTEURIZATION

Bacteriological studies of milk treated by the holder process have fixed the temperatures between 140° and 145° F. (60° to 62.8° C.), maintained for 30 minutes, as the minimum heating for the destruction of pathogenic organisms which may be found in milk. Although certain bacteria survive this heating it has been found that milk so treated is free from the ordinary disease-producing organisms, safe for consumption, unchanged in taste, and low enough in acid organisms to be handled without souring too quickly.

To study the effect of this process of pasteurization on mold spores, conidia from pure cultures of molds were first transferred to tubes of sterile water to obtain a suspension of spores. Transfers from such a suspension reduce the danger of such spores being blown by air currents into the cotton plugs and upon the walls of the test tubes used, where they might escape the full temperature applied to the milk. In the first series the inoculations were made by transferring 1 c. c. of this suspension in sterile pipettes into duplicate tubes of sterile milk. In a later series a platinum loop was used, since the tendency of the conidia to float thickly upon the surface of the water made this a quick and effective method of handling them. For most species it was thus possible to transfer spores enough to make a visible film over a part of the surface of the milk. None

of the species used produced visible growth except upon or near the surface of the milk. Observations of growth must include, therefore, the surface of the milk and especially the glass from the surface of the milk upward for a few millimeters, since most molds begin to grow first upon the glass. When no spores occurred upon the glass a free-floating colony in one case escaped observation until it fruited.

The inoculated milk tubes, with the exception of the control tubes, were heated in a water bath in which the water was agitated and the temperature of the milk was recorded in a control tube by a thermometer placed in the milk. The temperature in the tubes was not allowed to vary more than half a degree in either direction. The results of the experiments with the holder process are shown in Table I. In preparing this table the records of the checks, or unheated tubes, of successive experiments were found sufficiently uniform to permit them to be averaged and appear but once. Experimental tubes were made in duplicate; and when the results were not reasonably harmonious the work was repeated. Table I summarizes the tabulated data from a series of experiments extending over a period of several months.

TABLE I.—Comparative effect of heating mold spores in milk to temperatures of from 120° to 150° F. (48.9° to 65.6° C.) for 30 minutes¹

Name of mold.	Serial No.	Growth of spores when heated to temperature indicated and held for 30 minutes.											
		120° F. (48.9° C.)			125° F. (51.7° C.)			130° F. (54.5° C.)			135° F. (57.2° C.)		
		2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.
<i>Aspergillus candidus</i>	106	0.2	0.5	0.7	0.1	0.3	0.6	0.1	0.3	0.7	0.3	0.7	0.6
<i>Aspergillus flavus</i> (series).....	108	.4	.6	.9	.1	.4	.9	.5	.8	.5	.4	.9	.5
Do.....	ABC 3538	.5	.8	1.0	.1	.5	1.0	.5	.8	.8	.5	.8	.5
Do.....	Ra 136	.4	.9	1.0	.1	.5	1.0	.5	.8	.8	.5	.8	.5
Do.....	Sc 171	.4	.7	.9	.1	.5	1.0	.5	.8	.8	.5	.8	.5
<i>Aspergillus fumigatus</i>	118	.6	1.0	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Aspergillus globosus</i>	2795	.2	.4	.7	.2	.5	.8	.2	.5	.8	.2	.5	.8
Do.....	3512	.2	.5	.8	.2	.5	.8	.2	.5	.8	.2	.5	.8
Do.....	3555-21	.3	.9	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Aspergillus nidulans</i>	111	.4	1.0	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Aspergillus niger</i> (series).....	3536a	.4	.8	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Aspergillus niger</i> , var. <i>alipes</i>	3534b	.3	.7	1.0	.1	.4	.6	.1	.4	.6	.1	.4	.6
<i>Aspergillus niger</i> (cinnamomeus).....	3534c	.3	.7	1.0	.1	.4	.6	.1	.4	.6	.1	.4	.6
<i>Aspergillus niger</i> (lucens).....	112	.3	.7	1.0	.1	.4	.6	.1	.4	.6	.1	.4	.6
<i>Aspergillus ochraceus</i>	113	.4	.6	.9	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Aspergillus oryzae</i>	116	.2	.5	.8	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Aspergillus repens</i>	Ra 42	.2	.5	.8	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Aspergillus wentii</i>	3522-30	.3	.6	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Aspergillus</i> sp.....	3522-36	.3	.6	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
Do.....	3526	.3	.6	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
Do.....	3527	.3	.6	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Circinella</i> sp.....	3514 Cl	.3	.7	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Mucor</i> sp.....	3513	.3	.7	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
Do.....	3523-6	.3	.7	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
Do.....	3506	.3	.7	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
Do.....	3514 D4	.3	.7	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Rhizopus nigricans</i>	Do.....	.6	1.0	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Syncephalastrum</i> sp.....	Do.....	.6	1.0	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Fusarium</i> sp.....	Do.....	.3	.7	.9	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Oidium lactis</i>	Do.....	.3	.7	.9	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Penicillium atramentarium</i>	38	.2	.6	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7
<i>Penicillium brevicaulis</i>	2	.3	.6	1.0	.2	.4	.7	.3	.6	.3	.2	.4	.7

¹ 1.0, a typical spore-bearing colony; 0.1, discernible germination of conidia; tenths, 0.1 to 1.0, relative amount of growth; ?, doubtful; o, no growth; x, growth of a single spore; —, growth of a few widely scattered spores; +, growth of many spores; *, inharmonious results at times, but usually as given in the table.

TABLE I. — Comparative effect of heating mold spores in milk to temperatures of from 120° to 150° F. (48.9° to 65.6 C.) for 30 minutes—Continued

Name of mold.	Serial No.	Growth of spores when not heated (control).			Growth of spores when heated to temperature indicated and held for 30 minutes.																						
		2 days.	4 days.	6 days.	130° F. (48.9° C.).			125° F. (51.7° C.).			130° F. (54.5° C.).			135° F. (57.2° C.).			140° F. (60.0° C.).			145° F. (62.8° C.).			150° F. (65.6° C.).				
					2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.		
<i>Penicillium cankerberti</i>	5	0.2	0.3	0.5	0.1	0.3	0.6	(?)	0.3y	0.3	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium cankerberti</i> , var. <i>roqueforti</i>	6	0.2	0.4	0.7	0.2	0.4	0.8	(?)	0.2	0.3	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium chrysogenum</i>	26	0.3	0.7	1.0	0.3	0.7	1.0	0.1	0.4	0.8	(?)	(?)	0.2	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium citrinum</i>	15	0.2	0.5	0.8	(?)	0.0	0.0	(?)	0.3	0.4	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium commune</i>	23	0.2	0.4	0.8	(?)	0.3	0.4	(?)	0.4	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium cyclopium</i>	2543a	0.2	0.5	0.8	(?)	0.3	0.4	(?)	0.0	0.0	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium decarcatum</i>	14	0.3	0.4	0.6	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium expansum</i>	3523-4	0.3	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium granulosum</i>	9	0.2	0.4	0.7	(?)	0.3	0.4	(?)	0.3	0.4	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium italicum</i>	11	0.2	0.5	1.0	0.1	0.4	0.9	(?)	0.0	0.0	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium notatum</i>	102	0.2	0.5	1.0	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium pinophilum</i>	1	0.2	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium purpogenum</i>	17	0.3	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium roqueforti</i>	18	0.2	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium rugulosum</i>	46	0.2	0.5	0.7	0.2	0.4	0.8	(?)	0.3	0.4	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium solitum</i>	2546	0.2	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium spinulosum</i>	45	0.2	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium variable</i>	3551	0.2	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium viridicatum</i>	2643	0.2	0.4	0.7	0.1	0.4	0.8	(?)	0.3	0.4	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
Do.....	3028	0.3	0.6	0.9	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
Do.....	3514 Aa	0.2	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
<i>Penicillium asperulum</i>	2683	0.3	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
Do.....	28	0.3	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
Do.....	63	0.3	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
Do.....	103	0.3	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
Do.....	2670	0.2	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
Do.....	3525-6r	0.2	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
Do.....	3553	0.2	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
Do.....	3555-18	0.2	0.4	0.7	0.1+	0.4	0.8	(?)	0.1	0.5	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0
Do.....	3555-19	0.2	0.5	0.8	(?)	0.3	0.8	(?)	0.2	0.6	(?)	(?)	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	(?)	0.0	0.0	0.0	0.0

A study of Table I shows that very few mold spores survive exposure to 140° F. (60° C.) in milk for 30 minutes and that at 145° F. (62.8° C.) still fewer are found. With reference to significant organisms, among the mucors the *Mucor racemosus* group (3513, 3523.6, 3560) and *Rhizopus nigricans*, which are found more frequently than all others of this group combined, were destroyed at 130° F. (54.5° C.). The common green species of *Penicillium* are mostly dead at 130° F. (54.5° C.); a few stand 135° F. (57.2° C.), but two, one of them an undescribed soil organism, survived 140° F. (60° C.) for 30 minutes. Among species of *Aspergillus*, however, the strains of *A. flavus*, *A. fumigatus*, and *A. repens* all survived 145° F. (62.8° C.) for 30 minutes; *A. repens* and *A. fumigatus* both survived 150° F. (65.6° C.). These three species are always found in forage and feeding stuffs; hence, milk is more or less subject to contamination with them. *A. repens* grows very poorly in milk, however, and the examination of a great many cultures of milk and its products has shown that the actual development of *A. flavus* and *A. fumigatus* is comparatively rare. Although these organisms grow at blood heat and have demonstrated their pathogenicity even to human beings at rare intervals as causes of disease in the lungs, there is no report of their growth in the alimentary canal.

The destruction of mold spores by the holder process of pasteurization is shown more clearly in figure 1, where the results have been plotted.

Pasteurization of milk at 145° F. (62.8° C.) may therefore be regarded as destroying mold spores completely enough to render them a negligible factor in the further changes found in the milk.

EXPERIMENTS WITH THE FLASH PROCESS OF PASTEURIZATION

In working with continuous pasteurizers, temperatures of 165° to 175° F. (73.9° to 79.5° C.) are reached by heating within a period of approximately 30 seconds and maintained about 30 seconds. This is followed by quick cooling. Lower temperatures have not been deemed satisfactory. A series of experiments was therefore planned to subject the freshly inoculated spores of species of *Penicillium*, *Aspergillus*, and of the mucors to these temperatures and to determine their relative ability to survive such heating. For this purpose glass tubing about 3 mm. in diameter was drawn into capillary form so that each tube had 3 or 4 inches of the original tub-

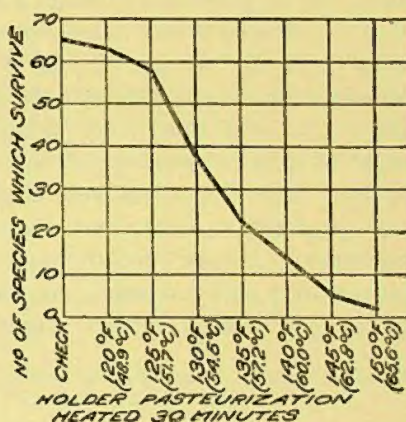


FIG. 1.—Curve of the number of species of molds surviving pasteurization of milk for 30 minutes at a series of temperatures.

ing with 2 to 4 inches of capillary tube approximately 0.5 mm. in diameter. The open end of each tube was plugged with cotton. The tubes were packed into a copper case and dry-sterilized. For each experiment a few drops of sterile milk were transferred to the conidial surface of a colony and the conidia stirred into the milk. A column of milk 15 to 30 mm. long, bearing numerous conidia, was then drawn into the capillary tube and the end sealed in the flame. Experiments had shown that alcohol boiling at 172.4° F. (78° C.) when so treated would boil in 20 to 30 seconds when the tubes were thrust into water at 174.4° F. (79.1° C.). This showed that milk containing mold spores could be heated in from 20 to 30 seconds in capillary tubes to any given temperature when immersed in water 2 degrees Fahrenheit above the desired pasteurizing temperature. In our experiments, therefore, it was possible to duplicate flash pasteurization on a laboratory scale; for example, to pasteurize at 165° F. (73.9° C.) the capillary tubes containing milk and mold spores were held in water at 167° F. (75° C.) for 1 minute. During this period about 30 seconds were required to heat the milk and it was held at the pasteurizing temperature the other half minute. This is approximately the heating period of milk in commercial flash pasteurization. After heating for the required time, the tubes were cooled by thrusting them into cold water. The tip of the capillary was then broken off and the contents streaked upon slanted Czapek's solution agar. The slants were incubated, observed occasionally, and the results of the various experiments were tabulated separately and then brought together in Table II.

TABLE II.—Comparative effect of heating mold spores in milk to temperatures of from 145° to 175° F. (62.8° to 79.5° C.) for 30 seconds¹

Name of mold.	Serial No.	Growth of spores.													
		Not heated (control).		Heated to 145° F. (62.8° C.).		Not heated (check).		Heated to 155° F. (68.3° C.).		Not heated (check).		Heated to 165° F. (73.9° C.).		Heated to 175° F. (79.5° C.).	
		6 days.	10 days.	6 days.	10 days.	3 days.	6 days.	3 days.	6 days.	4 days.	6 days.	4 days.	6 days.	4 days.	6 days.
<i>Aspergillus candidus</i>	106	0.4	0.7	0.8	1.0	0.3	0.7	0.0	0.0						
<i>Aspergillus flavus</i> series.....	108	.9	1.0	.8	1.0	.4	.8	.0	.0	0.6	1.0	0.0	0.0	0.0	0.0
Do.....	3538, 108	.9	1.0	.6	1.0	.4	.8	.0	.0						
Do.....	R2136	.8	1.0	.6	1.0	.5	.8	.0	.0						
Do.....	Sc171	.7	.9	.0	.0	.5	.9	.0	.0						
<i>Aspergillus fumigatus</i>	118	.9	1.0	.9	1.0	.3	.8	.0	.0	.6	1.0	.0	.0	.0	.0
<i>Aspergillus globosus</i> ?	2705	.8	1.0	.0	.0			.0	.0	.4	.8	.5?	1.0?	.0	.0
Do.....	3512					.3	.7								
Do.....	3555, 21	.8	.9	.0	.0	.3	.6	.0	.0						
<i>Aspergillus nidulans</i>	110	.3	1.0	.3	1.0	.3	.8	.0	.0	.5	.9	.0	.0	.0	.0
<i>Aspergillus niger</i>	111	.9	1.0	.0	.0	.0	.3	.7	.0	.6	1.0	.0	.0	.0	.0
<i>Aspergillus niger</i> , var. <i>altipes</i>	3534-a	.9	1.0	.6	1.0	.5	.8	?	.4?						
<i>Aspergillus cinnamomeus</i>	3534-b	.8	1.0	.6	1.0	.5	1.0	.0	.0	.5	1.0	.0	.0	.0	.0
<i>Aspergillus fuscus</i>	3534-c	.8	1.0	.7	.9			.0	.0	.7	1.0	.0	.0	.0	.0
<i>Aspergillus ochraceus</i>	112	.9	1.0	.0	.0	.9	1.0	.0	.0						
<i>Aspergillus oryzae</i>	113	.8	1.0	.0	.0	.9	1.0	.0	.0	.5	.8	.0	.0	.0	.0

¹ 1.0, a typical colony; decimals, proportionate growth; 0.0, no growth; ?, inharmonious results.

TABLE II.—Comparative effect of heating mold spores in milk to temperatures of from 145° to 175° F. (62.8° to 79.5° C.) for 30 seconds—Continued

Name of mold.	Serial No.	Growth of spores.													
		Not heated (control).		Heated to 145° F. (62.8° C.).		Not heated (check).		Heated to 155° F. (68.3° C.).		Not heated (check).		Heated to 165° F. (73.9° C.).		Heated to 175° F. (79.5° C.).	
		6 days.	10 days.	6 days.	10 days.	3 days.	6 days.	3 days.	6 days.	4 days.	6 days.	4 days.	6 days.	4 days.	6 days.
<i>Aspergillus repens</i>	146	.8	1.0	.0	.0	.8	1.0	.8	1.0	.0	.0	.0	.0	.0	.0
<i>Aspergillus wentii</i>	Ra42	.7	1.0	.6	1.0	.8	1.0	.0	.0	.5	.8	.0	.0	.0	.0
<i>Aspergillus</i> sp.....	3522-30	.9	1.0	.0	.0	.9	1.0	.0	.0	.0	.0	.0	.0	.0	.0
Do.....	3522-36	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
Do.....	3556	.9	1.0	.0	.0	.5	1.0	.0	.0	.0	.0	.0	.0	.0	.0
<i>Aspergillus parasiticus</i>	3509	.9	1.0	.6	1.0	.5	1.0	.0	.6?	.7	1.0	.0	.0	.0	.0
Do.....	3565	.9	1.0	.5	1.0	.7	.9	.0	.0	.0	.0	.0	.0	.0	.0
<i>Circinella umbellata</i>	3514-CI	.8	1.0	.8	1.0	.5	1.0	.0	.0	.8	1.0	.0	.0	.0	.0
<i>Mucor racemosus</i> (group).....	3513	.8	.8	.8	1.0	.9	1.0	.0	.0	.7	1.0	.0	.0	.0	.0
Do.....	3523-6	.0	.0	.0	.0	.9	1.0	.0	.0	.0	.0	.0	.0	.0	.0
Do.....	3560	1.0	1.0	1.0	1.0	.0	.0	.0	.0	.9	1.0	.0	.0	.0	.0
<i>Rhizopus nigricans</i>	3Ru.	.8	1.0	.0	.0	.6	1.0	.0	.0	.7	1.0	.0	.0	.0	.0
<i>Syncephalastrum</i> sp.....	Syn.	.9	1.0	.8	1.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
<i>Fusarium</i> sp.....	38	.8	1.0	.9	1.0	.5	1.0	.0	.7	.0	.0	.0	.0	.0	.0
<i>Penicillium atramentosum</i>	39	.9	1.0	.0	.0	.3	.6	.0	.0	.5	1.0	.0	.0	.0	.0
<i>Penicillium bisforme</i>	2	.3	bact.	.0	.0	.3	.6	.0	.0	.5	.6	.0	.0	.0	.0
<i>Penicillium brevicaulis</i>	5	.9	1.0	.0	.0	.9	1.0	.0	.0	.3	.7	.0	.0	.0	.0
<i>Penicillium camemberti</i> , var. <i>rogeri</i>	6	.8	1.0	.0	.0	.4	.6	.0	.0	.4	.9	.0	.0	.0	.0
<i>Penicillium chrysogenum</i>	26	.8	1.0	.0	.0	.4	.6	.0	.0	.4	.9	.0	.0	.0	.0
<i>Penicillium citrinum</i>	15	.7	1.0	.6	.7	.0	.0	.0	.0	.9	1.0	.0	.0	.0	.0
<i>Penicillium commune</i>	23	.8	1.0	.6	.8	.9	1.0	.0	.0	.7	1.0	.6?	1.0	.0	.0
<i>Penicillium cyclopium</i>	2543-a	.8	1.0	.4	.8	.5	.6	.0	.0	.5	1.0	.0	.0	.0	.0
<i>Penicillium digitatum</i>	16	.6	1.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
<i>Penicillium divaricatum</i>	34	.9	1.0	.9	1.0	.4	.6	.0	.0	.8	.9	.0	.0	.0	.0
<i>Penicillium duclauxi</i>	20	.8	1.0	.4	.5	.5	.9	.0	.0	.0	.0	.0	.0	.0	.0
<i>Penicillium expansum</i>	14	.9	1.0	.0	1.0	.5	.9	.0	.0	.4	.8	.0	.0	.0	.0
<i>Penicillium (Citromyces) sp.</i>	3523-4	.9	1.0	.0	1.0	.5	.9	.0	.0	.4	.8	.0	.0	.0	.0
<i>Penicillium granulosum</i>	9	.9	1.0	.0	.0	.5	.9	.0	.0	.4	1.0	.0	.0	.0	.0
<i>Penicillium italicum</i>	10	.4	.9	.5	1.0	.3	.6	.0	.0	.0	.0	.0	.0	.0	.0
<i>Penicillium luteum</i>	11	.8	.9	.4	1.0	.4	.8	Very slow.	.5	.9	.7	.0	.0	.0	.0
<i>Penicillium notatum</i>	102	.6	.7	.1	.5?	.3	.8	.0	.0	.3	.7	.0	.0	.0	.0
<i>Penicillium oxalicum</i>	103	.8	.9	.7	.7?	.5	.8	.4	.8	.9	.0	.0	.0	.0	.0
<i>Penicillium pinophilum</i>	1	.4	.8	.4	.8	.4	.9	.0	.0	.3	.7	.0	.0	.0	.0
<i>Penicillium puberulum</i> ?.....	2683	.8	1.0	.0	.0	.4	.9	.0	.0	.0	.0	.0	.0	.0	.0
<i>Penicillium purpurogenum</i>	17	.9	1.0	.0	.0	.3	.7	.0	.0	.6	.9	.0	.0	.0	.0
<i>Penicillium purpurogenum</i> , var. <i>rubri sclerotium</i>	2670	.0	.0	.0	.0	.3	.6	.0	.0	.5	1.0	.3	.5	.0	.6?
<i>Penicillium roqueforti</i>	18	.0	.0	.0	.0	.4	.8	.0	.0	.5	.9	.0	.0	.0	.0
<i>Penicillium rugulosum</i>	46	.4	.8	.4	.8	.0	.0	.0	.0	.3	.4	.0	.0	.0	.0
<i>Penicillium solitum</i>	2546	.8	1.0	.0	.0	.3	.6	.0	.0	.8	1.0	.0	.0	.0	.0
<i>Penicillium solitum</i> ?.....	66	.9	1.0	.6	1.0	.5	.9	.0	.0	.5	1.0	.0	.0	.0	.0
<i>Penicillium spinulosum</i>	45	.8	1.0	.7	.5	.0	.0	.0	.0	.5	.8	.0	.0	.0	.0
<i>Penicillium stoloniferum</i>	27	.9	1.0	.4?	1.0?	.4	.9	.0	.0	.5	.8	.0	.0	.0	.0
<i>Penicillium variable</i>	3551	.9	1.0	.0	.0	.3	1.0	.0	.7	.7	.8	1.0	.0	.0	.0
<i>Penicillium viridicatum</i>	2552	.9	1.0	.7	.6	.3	.9	.0	.0	.0	.0	.0	.0	.0	.0
<i>Penicillium viridicatum</i> , var. ?.....	2643	.9	1.0	.7	.8	.3	.7	.0	.0	.5	.7	.0	.0	.0	.0
Do.....	3028	.7	1.0	.0	.0	.3	.8	.0	.0	.7	1.0	.8?	.9?	.0	.0
Do.....	1514-a	.8	1.0	.4?	.6?	.4	.6	.0	.0	.5	1.0	.0	.0	.0	.0
<i>Penicillium (Citromyces) sp.</i>	28	.0	.0	.6	.8	.3	.6	.0	.0	.4	.9	.0	.0	.0	.0
Do.....	63	.9	1.0	.0	.0	.4	.7	.0	.0	.5	1.0	.0	.0	.0	.0
<i>Penicillium</i> sp.....	3525-61	.8	1.0	.7	.4	.4	.8	.0	.0	.6	.9	.0	.0	.0	.0
Do.....	3553	.0	.0	.0	.0	.5	.9	.0	.0	.4	.7	.0	.0	.0	.0
Do.....	3555-18	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
Do.....	3555-19	.7	.9	.4	.7	.6	.9	.0	.0	.0	.0	.0	.0	.0	.0

From Table II it is seen that very few of the forms are killed in 30 seconds at 145° F. (62.8° C.); nearly all, however, are destroyed at 155° F. (68.3° C.). None of the colonies found at 165° F. (73.9° C.) and 175° F. (79.5° C.) were produced in both tubes. The chance of error is not fully eliminated in these cases. The consistent character of the whole table and the innocuous character of the few organisms in which occasional colonies occurred after heating show that temperatures of 165° to 175° F. (73.9° to 79.5° C.) for 30 seconds do practically destroy the spores of these molds as they may be found in milk, although a few

conidia in some species may occasionally survive.

Figure 2 shows graphically the effect of the flash process of pasteurization on mold spores.

DESTRUCTION OF MOLD SPORES BY DRY HEAT

The third series of experiments was planned to find the relative ability of the spores of approximately the same organisms to endure heating in dry air for the same period as used for heating in milk. After some experimentation the following method was used: Strips of

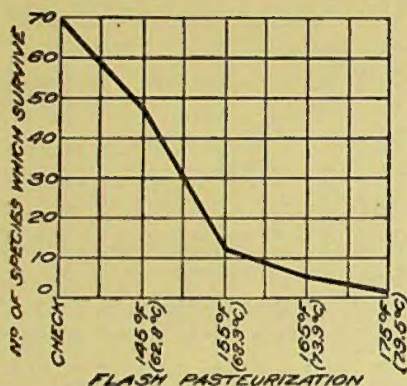


FIG. 2.—Curve of the number of species of molds surviving flash pasteurization at a series of temperatures.

heavy filter paper were cut wide enough so that only the edges would come into contact with the glass when dropped into test tubes. A drop of sterile water carrying a suspension of the spores under experiment was deposited in the middle of the paper strip and allowed to evaporate overnight. The tubes were then immersed in liquid heated to the desired temperature and held 30 minutes after check tubes carrying thermometers indicated that the air in the tubes had reached the same degree. The tubes were then removed and cooled. Melted agar was allowed to run into each tube to form a slant and the cultures were set away at room temperature. Observations of growth were made as in the previous experiments and the results tabulated in the same manner in Table III.

TABLE III.—Comparative ability of mold spores to survive heating in dry air for 30 minutes at temperatures of 180° to 250° F. (82.2° to 121.1° C.)¹

Growth of spores when not heated (control) and after having been heated to the temperature indicated for 30 minutes.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
Name of mold.	Serial No.	Not heated (con- trol) 5 days.		Heated to 180° F. (82.2° C.) 5 days.		Heated to 190° F. (87.8° C.) 3 days.		Heated to 190° F. (87.8° C.) 7 days.		Not heated (con- trol) 4 days.		Heated to 200° F. (93.3° C.) 4 days.		Heated to 200° F. (93.3° C.) 8 days.		Not heated (con- trol) 4 days.		Heated to 210° F. (98.9° C.) 4 days.		Heated to 210° F. (98.9° C.) 7 days.		Not heated (con- trol) 3 days.		Heated to 220° F. (104.5° C.) 4 days.		Heated to 220° F. (104.5° C.) 7 days.		Not heated (con- trol) 6 days.		Heated to 230° F. (110.0° C.) 3 days.		Heated to 230° F. (110.0° C.) 6 days.		Not heated (con- trol) 4 days.		Heated to 250° F. (121.1° C.) 4 days.		Heated to 250° F. (121.1° C.) 8 days.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
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¹ i. o., a typical colony; decimals, proportionate growth, o. o., no growth; ? inharmonious results; y, growth of a single spore.

A study of Table III shows that mold spores possess much greater ability to withstand dry heat than heating in milk. Very few forms were destroyed at 180° F. (82.2° C.), but they include *Penicillium brevicaulis*, which has a thick-walled spore and in laboratory cultures has remained viable at least 7 years. Only a few species of *Penicillium* survived heating to 200° F. (93.3° C.) for 30 minutes. All these are forms which grew at 98.6° F. (37° C.), and some of them are widely distributed.

Aside from *A. wentii*, all the species of *Aspergillus* survived heating at 200° F. (93.3° C.). Several of them survived at 230° F. (110° C.), but after 250° F. (121.1° C.) for 30 minutes no species showed growth after 6 days' incubation. Three of six mucors, however, survived the heating to 250° F. (121.1° C.) for 30 minutes. These species were killed

quickly by both forms of heating in milk. The results of these experiments are plotted in figure 3.

The destruction of mold spores by dry heat has no relation to the subject of pasteurization of milk, but it is of scientific interest.

DISCUSSION OF RESULTS

These results with mold spores agree in general with bacteriological studies of pasteurization. Very few of these organisms found in milk survive after 30 minutes' heating to 145° F. (62.8°

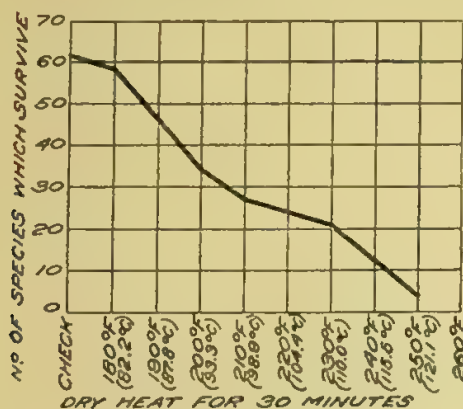


FIG. 3.—Curve of the number of species of molds surviving dry heat for 30 minutes at a series of temperatures.

C.). Certain molds, notably *Aspergillus fumigatus* and *A. flavus*, do survive, but they occur only occasionally in milk. *Oidium lactis* and the mucors, which are probably more important as milk-borne organisms than all the rest, are destroyed at the low temperatures used in the holder process of pasteurization. In the flash process very few mold spores survived at 165° F. (73.9° C.). Occasionally some spores seem to have escaped destruction at 175° F. (79.5° C.), but the organisms surviving in these cases were of minor importance in the decomposition of dairy products. In confirmation of these results the writers have had access to unpublished data of Mr. R. O. Webster, of the Bureau of Chemistry, giving cultural analysis of butter made from flash-pasteurized cream on a commercial basis. Cultures from this butter showed no mold spores, while cultures made at the same time from country butter showed 20,000 to 60,000 per gram.

Mold spores in milk seem, therefore, to be destroyed completely or reduced to negligible numbers by both of the standard pasteurization processes.

Careful study of the cultures showed that the first effect of heating was to delay germination. This is indicated in the tables by the reports of successive examinations of the same culture. In Table I three reports are given; later only two reports. The third and fourth observations, however, were usually made. At times heating to a degree just under the death point delayed germination almost the full length of the usual growing period of the species. The number of possible sources of error was so great that the results of observations have been tabulated and compared. When essential harmony of results was not obtained, the work was repeated. In a few cases the continued lack of consistent results for particular organisms is indicated by the interrogation point in the tables. Even with these precautions the data obtained can be said to apply only to the strains used. This is indicated by comparing the results given for the *Aspergillus flavus* group or for the four members of the *A. niger* group. These results do not prove that other strains of these groups would respond exactly as here tabulated. In fact, more extended studies (as yet unpublished) of these two groups indicate that organisms otherwise undistinguishable may differ greatly if we measure a single physiological reaction. Such quantitative differences may persist in continued cultures, but are hardly comparable to differences in the kind of reaction as a basis for separating species. Inside the race or strain, conidia transferred from the same culture respond very differently. There is frequently a survival of a few spores where a majority of the spores die. There may be, therefore, a difference of as much as 20° F. (11.1 C.) between the temperature at which an occasional culture is completely killed and that at which cultures of that species are uniformly killed. These results resemble those obtained in determining the thermal death point of bacteria.

The applicability of these results to the occurrence of mold spores in substances other than milk has not been tested. The variation in composition of the substratum together with the heating may at times introduce a considerable variation. In general, however, it is clear that mold spores are easily killed by heat when suspended in fluid. The tables have been studied in an attempt to correlate resistance with size of spore or thickness of spore wall. No such correlation has been found. There is, therefore, no suggestion as to the nature of the difference in these organisms which affects their resistance to heat.

SUMMARY

(1) The holder process of pasteurization, in which milk was heated to 145° F. (62.8° C.) and maintained at that temperature for 30 minutes, killed the conidia of every species investigated, except those of *Asper-*

gillus repens, *A. flavus*, and *A. fumigatus*. The molds which survive are found only occasionally in milk.

(2) The flash process of pasteurization, where milk was heated to 165° F. (73.9° C.) for a period of 30 seconds, destroyed the spores of all the molds tested with the exception of many spores of one form and occasional spores of three more forms. At 175° F. (79.5° C.) only occasional spores of two forms developed.

(3) When the heating process was performed in dry air for a period of 30 seconds at 200° F. (93.3° C.), 31 out of 42 forms of *Penicillium* and 7 out of 24 forms of *Aspergillus* were destroyed, but none of the cultures of the mucors. A temperature of 250° F. (121.1° C.) over a period of 30 minutes killed all the forms of *Penicillium* spp. tried, but left an occasional living spore in one species of *Aspergillus* and three out of six mucors.

